

## Specific regulation of *c-myc* oncogene expression in a murine B-cell lymphoma

(WEHI 231/immune regulation/RNA turnover/translational control)

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**ABSTRACT** The *c-myc* oncogene has been implicated in a wide spectrum of B-cell neoplasias. In normal cells, the level of expression of the *c-myc* gene correlates with growth status. In the present study, we examined the effect of receptor-mediated inhibition of growth on *c-myc* expression in a B-cell lymphoma. The murine lymphoma line WEHI 231 has been characterized as an early B cell; it bears surface-bound IgM and has unrearranged *c-myc* genes. Following treatment of a WEHI 231 culture with anti-mouse Ig antiserum, the cells undergo one round of division and further proliferation is inhibited. We observed that this treatment specifically affected cytoplasmic levels of *c-myc* mRNA. An initial early increase is followed by a precipitous drop such that by 4 hr (after exposure) the amount of *c-myc* mRNA is below control values by a factor of  $\approx 10$ . The drop in *c-myc* precedes cessation of DNA synthesis. During the 2- to 4-hr period, *c-myc* mRNA had a maximal half-life of between 20 and 30 min. In contrast, even 24 hr after anti-Ig exposure, the amounts of most major mRNAs, including  $\mu$  heavy chain and actin, were not significantly altered. These results indicate that expression of an unrearranged *c-myc* gene can be selectively responsive to receptor-mediated regulatory events.

The response pattern of a B lymphocyte to a particular antigenic signal depends upon many variables, including the stage of differentiation of the cell itself and the presence or absence of ancillary signals such as T-cell factors. The primary B-cell receptor is the specific Ig produced by the cell that becomes integrated within the cell surface membrane (surface-bound Ig, sIg). Cross-linking of sIg may result in either stimulation or inhibition of further B-cell development (1-5). The interaction of mature B cells with antigen, along with factors derived from helper T cells, triggers the clonal expansion of antigen-specific B cells and the differentiation of those cells to Ig-secreting plasma cells (6, 7). Conversely, it is postulated that binding of antigen to sIg of developmentally early B cells causes the cessation of cell growth, leading to immunological tolerance (3, 8). Little is known of the metabolic events that underlie this regulation of B-cell growth and differentiation.

WEHI 231 is a murine lymphoma line that has been characterized as an early B cell on the basis of surface markers and biological properties (9). Although transformed, these cells remain capable of responding to various immunological stimuli in a manner reminiscent of nontransformed cells, suggesting that they may serve as a useful model for growth control and differentiation of normal B cells. For example, they normally express IgM while secreting only very low amounts of Ig into the medium. Upon stimulation with bacterial lipopolysaccharide, which has been shown to promote B-cell differentiation, they begin secreting pentameric IgM,

while levels of surface IgM decrease (10). Treatment of WEHI 231 with anti-mouse Ig antiserum inhibits proliferation of these cells. The growth inhibition has been shown to be mediated specifically by binding of antibody to surface IgM since it can be effected by a monoclonal anti-mouse IgM antibody, the F(ab')<sub>2</sub> portion of this antibody fraction, or the affinity-purified anti-mouse IgM fraction of a rabbit antiserum prepared against mouse Ig (8). Based on these observations, Boyd and Schrader (8) have proposed that WEHI 231 represents a model system for investigating the mechanism by which interaction with sIg receptor results in inhibition of B-cell growth, and possibly a model system for the study of tolerance.

The proto-oncogene *c-myc* is a normal cellular gene that has been conserved during evolution (11) and is expressed in all tissue types examined (12). Perturbations of the *c-myc* locus have been documented in many B-cell malignancies of human, mouse, and chicken origin. These alterations include the nearby integration of a retrovirus (13), point mutations (14), and chromosomal translocations (15-17). In addition, *c-myc* sequences are present in regions of amplified DNA in several human tumors (18). The *c-myc* protein has been localized to the nuclear matrix (19), although its function is not understood. We and others have shown that in nonneoplastically transformed cells the level of *c-myc* mRNA is low during quiescence and that it increases 10- to 30-fold as the cells begin growing (20, 21). During differentiation of F9 teratocarcinoma cells to nonproliferating endoderm cells, expression of *c-myc* message decreases upon terminal differentiation. This evidence suggests that the *c-myc* gene is involved in some aspect of regulation of cell proliferation.

We have examined the effect of *in vitro* anti-sIg treatment on gene expression in WEHI 231 cells. Following exposure to anti-mouse Ig antiserum, cells underwent one round of division; growth was arrested by 24 hr. Although levels of most mRNA species remained unchanged during the time period, cytoplasmic *c-myc* mRNA levels increased quickly and then decreased very suddenly. These results indicate that the *c-myc* gene is rapidly and selectively controlled after antibody binding to the IgM receptor, and its expression is under both positive and negative regulation.

### METHODS

**Cell Culture and Antibody Treatment.** In these studies we used a variant of WEHI 231 (kindly provided by L. Wysocki), selected for sensitivity to HAT (hypoxanthine/aminopterin/thymidine) medium. Cells were maintained in Dulbecco's modified Eagle's medium with 20% fetal calf serum supplemented with 0.058% glucose, 0.35% glutamine, nonessential amino acids, and 50  $\mu$ M 2-mercaptoethanol. Cultures were incubated at 37°C in a 5% CO<sub>2</sub> in air mixture.

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Abbreviation: sIg, surface-bound Ig.

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Prior to antibody treatment, cells were grown to a density of  $5-10 \times 10^5$  cells per ml, pelleted and resuspended in fresh, prewarmed medium at  $1-5 \times 10^5$  cells per ml, and allowed to incubate for a minimum of 1–2 hr or as long as overnight. Several dilutions of filter-sterilized goat anti-mouse Ig serum (kindly provided by M. Mage, National Cancer Institute, National Institutes of Health) were tested to determine the minimal level required for inhibition of cell growth. Cell growth was reproducibly arrested within 24 hr with a 1:500 dilution of this antiserum. Cell death was not immediate since the inhibition could be reversed upon removal of the antiserum even after 48 hr of treatment. Higher concentrations of antiserum resulted in an increasing degree of cell death, as has been reported (8).

**Incorporation of [ $^3\text{H}$ ]thymidine.** Aliquots (0.2 ml) of control or antibody-treated WEHI 231 cells were removed, in triplicate, at various times and transferred to microtiter wells. To each well, 0.5  $\mu\text{Ci}$  (1 Ci = 37 GBq) of [ $^3\text{H}$ ]thymidine (6–10 Ci/mmol, ICN) was added in 10  $\mu\text{l}$  of Hanks' buffered salt solution (Flow Laboratories). After 1 hr of incubation, cells were harvested on glass fiber filters, lysed, and washed with distilled water using a PHD cell harvester (Cambridge Technology, Cambridge, MA). The levels of incorporated [ $^3\text{H}$ ]thymidine were measured in an LKB rack beta scintillation counter.

**RNA Analysis.** Poly(A)-containing cytoplasmic RNA was isolated, denatured, electrophoresed on formaldehyde/agarose gels, transferred to nitrocellulose paper, and hybridized to  $^{32}\text{P}$ -labeled probes as described (22). Relative hybridization intensities were evaluated by scanning densitometry and integration of the peak areas. Quantitation of poly(A) levels was made by using hybridization to [ $^3\text{H}$ ]poly(U) (21). The *c-myc* sequence probe (kindly provided by R. Taub and P. Leder) was prepared from the *c-myc*-excluded  $C_\alpha$  allele locus of the IgA-producing mouse myeloma S107 and cloned into pBR322 (22). A genomic clone of the  $\mu$  heavy chain ( $C_{H\mu}7$ ) was kindly supplied by L. Hood. The actin probe was a 1.0-kilobase cDNA clone of rat actin, generously provided by S. Farmer. The *c-Ki-ras* probe, a subclone of the Kirsten (HiHi3) murine sarcoma virus, was a gift from E. Scolnick.

Cell-free translation of total cytoplasmic RNA in a rabbit reticulocyte lysate, pretreated with micrococcal nuclease, was performed as described (23). The L-[ $^{35}\text{S}$ ]methionine-labeled polypeptides were reduced with 2-mercaptoethanol, separated on 6–11% linear gradient polyacrylamide/NaDodSO<sub>4</sub> gels, and fluorographed as described (23).

**Protein Synthesis and Polysome Analysis.** At various time points, aliquots of control or antibody-treated cells were removed and incubated with 20  $\mu\text{Ci}$  of L-[ $^{35}\text{S}$ ]methionine (1100 Ci/mmol; Amersham) per ml for 20 min. Following harvesting, cells were resuspended in 50 mM NaCl/50 mM Tris·HCl, pH 7.6, and lysed by the addition of Triton X-100 and sodium deoxycholate to 1% final concentrations, and the nuclei were removed by centrifugation. Aliquots were removed and incorporation of radiolabel was measured following precipitation with trichloroacetic acid. Additional samples were analyzed by NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis as above.

For analysis of polyribosome structures, control or antibody-treated cultures were chilled rapidly on ice, cells were harvested, cytoplasmic lysates were prepared, and polysomes were separated in 10–40% sucrose (50 mM Tris·HCl, pH 7.6/100 mM NaCl/1 mM MgCl<sub>2</sub>) gradients as described (22).

## RESULTS

**Treatment of WEHI 231 Cells with Anti-Ig Antiserum Inhibits Cell Division.** To monitor the kinetics of growth inhibition, we evaluated the effect of anti-Ig antiserum treatment

Table 1. Anti-Ig treatment of WEHI 231 cells inhibits cell growth and DNA synthesis

Time after antibody addition, hr	Cells, no. per ml	$^3\text{H}$ , cpm per $10^5$ cells
0	$4.8 \times 10^5$	2359
8	$1.0 \times 10^6$	5382
24	$1.2 \times 10^6$	490
46	$9.7 \times 10^5$	648
0	$4.3 \times 10^5$	2265
8	$6.2 \times 10^5$	5625
24	$8.3 \times 10^5$	486
46	$8.3 \times 10^5$	413

on cell doubling and DNA synthesis (Table 1). The normal doubling time of WEHI 231 cells under these tissue culture conditions is between 16 and 18 hr. With anti-Ig treatment cell numbers had approximately doubled by 24 hr and remained the same after an additional 22 hr. Extensive cell debris was apparent at this time. The level of [ $^3\text{H}$ ]thymidine incorporation during a 1-hr pulse period in control and anti-Ig-treated cultures was determined at 8, 24, and 46 hr. The results of two separate experiments are shown in Table 1. DNA synthesis continued for at least 8 hr. By 24 hr, the incorporation of [ $^3\text{H}$ ]thymidine in treated cultures was decreased by 80% compared to the level in control cultures. In a separate series of experiments, equal aliquots of the culture were removed 8, 12, 16, 20, and 24 hr after initiation of antibody treatment and were pulse-labeled with [ $^3\text{H}$ ]thymidine as above. A decline in the cellular rate of DNA synthesis was first observed at 20 hr and the decrease continued until a value of under 20% of control levels was reached by 24 hr (data not shown). Thus, following antibody treatment, the cells undergo one round of division and then further growth is inhibited.

**Treatment with Anti-Ig Antiserum Results in Selective Changes of *c-myc* mRNA Levels.** The effect of treatment with anti-Ig antiserum on levels of cytoplasmic mRNA was evaluated. Cells were harvested after 0, 3, 6, and 24 hr of treatment. Cytoplasmic RNA was extracted and poly(A)-containing RNA was isolated by oligo(dT)-cellulose chromatography. No significant alteration in the level of total or poly(A)-containing mRNA was detected with anti-Ig treatment (data not shown).

Three specific messages were then chosen for further study: *c-myc* oncogene,  $\mu$  heavy chain of IgM, and actin. Equal amounts of poly(A)-containing RNA from control and treated cells, isolated at 2.5, 6, and 25 hr, were electrophoresed on a formaldehyde/agarose gel and transferred to cellulose nitrate paper; the resulting blot was hybridized to a  $^{32}\text{P}$ -labeled mouse *c-myc* probe (Fig. 1A). Parallel control cultures were monitored to evaluate whether the level of cytoplasmic *c-myc* mRNA would vary during the course of the incubation in the absence of antibody treatment. Levels of *c-myc* remained essentially unchanged in control cells. In contrast, following exposure to anti-Ig antiserum, the level of *c-myc* RNA underwent a rapid increase followed by a dramatic decrease to an amount significantly below control levels. Although accurate quantitation by densitometric scanning of data with such vast fluctuations is difficult, an increase over control levels of  $\approx 10$ -fold by 2½ hr and a decrease by a factor of 10 below control values by 24 hr was estimated.

To examine the specificity of the changes, the mRNA levels of  $\mu$  heavy chain (Fig. 1B) and actin (Fig. 1C) were measured. The  $\mu$  heavy chain was chosen as a message representative of the specialized function of WEHI 231 lymphoma cells (i.e., an Ig chain), whereas actin was chosen as a message coding for a general "housekeeping" protein expressed in all cell types. Hybridization to a  $\mu$  constant region probe

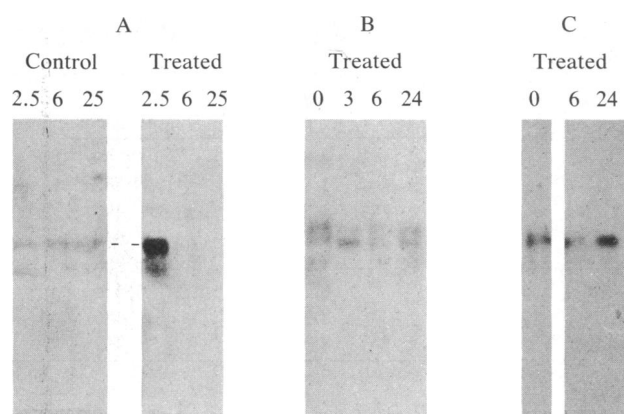


FIG. 1. Treatment of WEHI 231 cells with anti-mouse Ig serum specifically affects *c-myc* mRNA levels. Exponentially growing WEHI 231 cells were either mock-treated (control) or treated with a 1:500 dilution of goat anti-mouse Ig serum. Cells were harvested at the indicated times (hours), and poly(A)-containing cytoplasmic RNA was isolated. For each time point, equal quantities of denatured RNA were electrophoresed, transferred to cellulose-nitrate paper, and hybridized to the indicated  $^{32}\text{P}$ -labeled DNA probe. (A) *c-myc*: the position of the *c-myc* mRNA is as indicated by the line (2  $\mu\text{g}$  per lane); (B)  $\mu$  heavy chain: hybridization to the mRNA species for the secretory (bottom) and membrane-associated (upper) heavy chain is detected (5  $\mu\text{g}$  per lane); (C) actin (2  $\mu\text{g}$  per lane).

detected two mRNA species (Fig. 1B). Presumably, the upper band is for the membrane-associated  $\mu$  heavy chain and the lower band is for the secretory form of this heavy chain polypeptide, as has been shown previously (24). With antibody treatment, no major changes were observed in the total amounts of heavy chain mRNA; however, a shift was detected in the relative quantities of the two forms. It was not determined whether the cells were triggered to secrete IgM by this treatment. It should be noted that control cells do not secrete significant levels of IgM even though  $\approx 30\%$  of the mRNA has been apparently processed to the secretory form (unpublished observation). Moreover, antibody treatment did not significantly alter the amounts of poly(A)-containing cytoplasmic messages for actin (Fig. 1C), as judged by the 6- and 24-hr time points tested.

To further evaluate the effect of anti-Ig treatment on the overall mRNA population, translation in a cell-free system was performed. Equal amounts of total cytoplasmic RNA (10  $\mu\text{g}$ ) were used to direct the synthesis of proteins in a rabbit reticulocyte cell-free system, and the [ $^{35}\text{S}$ ]methionine-labeled products were fractionated on a NaDodSO<sub>4</sub>/polyacrylamide gel (Fig. 2A). The overall protein profile observed with RNA isolated after 2, 6, or 24 hr of treatment changed very little compared to that obtained with control RNA. (Although not well resolved in this particular gel, it should be noted that one minor band at 28 kDa does appear to be increased in the 24-hr sample.) Thus, though antibody treatment of WEHI 231 cells has little effect on the levels of most cytoplasmic mRNAs, dramatic alterations of expression of *c-myc* mRNA are observed.

**Levels of *c-myc* mRNA Fluctuate Rapidly, Whereas *c-Ki-ras* mRNA Levels Remain Essentially Unaltered.** To look at the early events in more detail, RNA was isolated after 0, 2, 4, and 6 hr of antibody treatment. Equal amounts of poly(A)-containing RNA were analyzed from each time point (Fig. 3A). The autoradiograms from this and duplicate blots were scanned to evaluate the quantity of *c-myc* mRNA in treated cells compared to control cultures. An approximately 5- to 10-fold increase in *c-myc* RNA levels was observed by 2 hr. A very rapid decrease in the amount of *c-myc* mRNA (by a factor of 25–50) then occurred within the next 2 hr. As con-

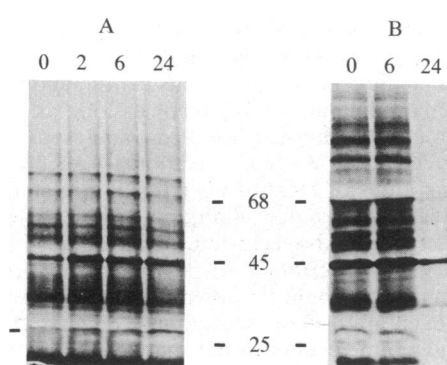


FIG. 2. Anti-Ig treatment has little effect on levels of most major mRNA species but inhibits protein synthesis. (A) Cell-free translation analysis of RNA. Equal quantities of cytoplasmic RNA (10  $\mu\text{g}$ ) from control or Ig-treated cultures, isolated at the indicated times (hours), were translated in micrococcal nuclease-treated rabbit reticulocyte lysates. The products, labeled with L-[ $^{35}\text{S}$ ]methionine, were electrophoresed on NaDodSO<sub>4</sub>/polyacrylamide gels and the resulting fluorogram was obtained. Under these conditions incorporation into endogenous polypeptides is barely detectable (data not shown). The positions of marker proteins of 25, 45, and 68 kDa are as shown; the expected position of a 28-kDa polypeptide is indicated by the line. (B) Intracellular protein synthesis is inhibited. Control or Ig-treated cultures were initiated at a concentration of  $1 \times 10^5$  cells per ml. Equal aliquots, removed at the indicated times (hours), were pulse-labeled with L-[ $^{35}\text{S}$ ]methionine for 20 min. Equal cell equivalents of cytoplasmic lysates were analyzed, as above. The expected position of a 35-kDa protein is indicated by the line.

trol, actin levels were again monitored. No significant changes in actin levels were observed over the time course of the experiment (data not shown).

To assess the role of anti-sIg treatment on another oncogene whose expression correlates with cell proliferation, *c-Ki-ras* mRNA production was also monitored (Fig. 3B). In contrast to the *c-myc* oncogene, expression of the *c-Ki-ras* oncogene is growth related even in chemically transformed 3T3 cells; *c-Ki-ras* mRNA levels were observed to increase

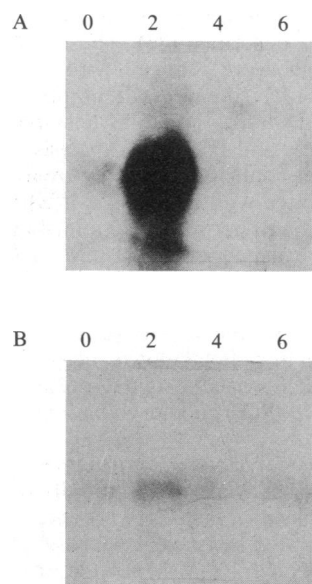


FIG. 3. Changes in *c-myc* mRNA levels occur rapidly. Equal amounts of poly(A)-containing RNA (4  $\mu\text{g}$ ) from control or Ig-treated WEHI 231 cells were isolated at the indicated times (hours) and assessed by RNA blot analysis as in Fig. 1. (A) Hybridization to  $^{32}\text{P}$ -labeled *c-myc* probe; (B) hybridization to  $^{32}\text{P}$ -labeled *c-Ki-ras* probe.

5- to 7-fold in growing cells compared to quiescent cells (21). After 2 hr of antibody treatment, the level of *c-Ki-ras* mRNA appeared to increase slightly (<2-fold) and then returned to control levels. [In other experiments, the levels of *c-Ki-ras* were essentially unchanged at the 3- and 6-hr time points (data not shown).] Thus, the rapid plummet in *c-myc* mRNA is selective; the expression of other mRNAs and another growth-regulated oncogene is not significantly affected by the antibody treatment.

**Protein Synthesis is Inhibited by Anti-Ig Treatment.** To monitor the effect of anti-Ig treatment on protein synthesis, the incorporation of [<sup>35</sup>S]methionine into protein was measured. Control and anti-Ig-treated WEHI 231 cells were incubated for various periods of time and then pulsed for 20 min in medium containing [<sup>35</sup>S]methionine. Cytoplasmic lysates, analyzed for incorporation of radiolabel into acid-precipitable material, showed an approximate 75% decrease in [<sup>35</sup>S]methionine incorporation per cell following 24 hr of anti-Ig treatment (data not shown). This result suggests that the rate of protein synthesis is greatly diminished following incubation with anti-Ig antiserum. However, since any alteration in the methionine pool size would severely alter these measurements, the polyribosome profiles of control and 24-hr treated cells were investigated as an independent test of the effect of anti-Ig on protein synthesis. Treated and untreated WEHI 231 cells were lysed, the crude polysomes were fractionated over sucrose gradients, and the gradients were collected with continuous ultraviolet monitoring at O.D.<sub>254</sub>. Though the profile of the control cells displayed the normal pattern of large and small polysomes, the 24-hr treated culture contained very few ribosomes migrating in the polysome regions of the gradient (data not shown), indicating that translation of mRNAs was severely inhibited 24 hr after anti-sIg treatment.

The pattern of proteins produced by cells after various times of treatment was monitored. Aliquots of cell lysates, normalized for equal cell numbers, were electrophoresed on a NaDodSO<sub>4</sub>/polyacrylamide gel (Fig. 2B). A general decrease in incorporation of [<sup>35</sup>S]methionine into proteins was visible after 24 hr of treatment, although the synthesis of a few polypeptides seemed to be more resistant to this inhibitory effect. Polypeptide bands migrating at approximately 68, 44, and 35 kDa are visible. The mRNAs for most polypeptides have been presumably stored within the cytoplasm (possibly as messenger ribonucleoprotein particles), since equal levels of translationally active poly(A)-containing mRNA can be extracted from control and 24-hr treated cultures (see above). Thus, although most mRNAs are present in the anti-Ig-treated cells, their intracellular translational activity is greatly reduced.

## DISCUSSION

Cultures of exponentially growing WEHI 231 cells treated with an anti-mouse Ig antiserum cease proliferation by 24 hr after completing one round of cell division. This arrest of growth by a specific receptor-mediated signal is preceded by a dramatic decline in the cytoplasmic levels of *c-myc* mRNA. The fluctuations in *c-myc* are selective in that significant changes in the levels of other mRNA species were not observed. Furthermore, the drop in *c-myc* mRNA occurs well before inhibition of DNA synthesis is evident; DNA synthesis continues for ≈16–20 hr in the presence of decreased *c-myc* mRNA levels. It has been found that as a result of this treatment, WEHI 231 cells arrest in the G<sub>1</sub> phase of the cell cycle (A. DeFranco, personal communication). These results extend previous observations on the growth-related expression of *c-myc* mRNA, in which the levels of *c-myc* message were found to be lower in quiescent BALB/c 3T3 fibroblasts (20, 21) or terminally differentiated endo-

derm cells than in the actively dividing fibroblasts or F9 stem cells, respectively (21), and the observation that mitogens that stimulated DNA replication resulted in an early and dramatic increase in *c-myc* mRNA levels (20).

During the initial phase of the treatment, an approximate 5- to 10-fold increase in cytoplasmic *c-myc* mRNA levels occurs. Questions remain as to whether this increase is necessary for the inhibition of growth or results from some form of initial activation of the B cell prior to suppression. The *c-myc* mRNA appears functional as it has been localized within polyribosome structures (25). Similar rapid enhancement of *c-myc* transcripts has been observed in resting B or T cells after mitogen stimulation (20) and in quiescent BALB/c 3T3 fibroblast cells following exposure to serum or platelet-derived growth factor (20, 21). This increase occurs early in the G<sub>0</sub>/G<sub>1</sub> period of the cell cycle (20, 21). In the present experiments, the WEHI 231 cultures were not synchronized prior to initiation of treatment; therefore, an obvious experiment is to determine whether this *c-myc* activation can occur within cells at any stage of the cell cycle.

The results of our experiments indicate that *c-myc* gene expression is subject to stringent negative as well as positive regulation. Such tight regulation is unusual and suggests an important role for *c-myc* in cell proliferation. Though it is most likely that increased transcription is responsible for the enhanced levels of *c-myc* mRNA, the possibility of altered processing has not been eliminated. For the decrease in *c-myc* mRNA levels, inhibition of mRNA production alone is probably not sufficient to explain the dramatic decline. Thus, we propose that a specific turnover mechanism may play a role. Between 2 and 4 hr following initiation of anti-sIg treatment, the drop in *c-myc* mRNA levels was by a factor of 25–50. From these values an approximate half-life of between 20 and 30 min can be derived. The degradation appears specific for *c-myc* mRNA since other messages were not significantly affected by anti-sIg treatment. In addition, the decrease is much more dramatic than that observed for the decline in *c-myc* RNA levels following mitogen stimulation of B or T cells or during terminal differentiation of F9 teratocarcinoma cells (20, 21). Thus, although synthesis of *c-myc* mRNA may be inhibited between 2 and 4 hr of anti-sIg treatment, the data suggest that during this period the production of some factor involved in *c-myc* mRNA degradation predominates. This could be new synthesis of a protein factor (or activation of a pre-existing factor) that recognizes some specific sequence within the *c-myc* mRNA and focuses a ribonuclease activity. Alternatively, a ribonuclease itself may be synthesized that specifically recognizes some structural feature of the *c-myc* mRNA (26). Similar half-lives have been measured for some of the histone mRNAs (27) that are controlled at multiple levels, including transcription and RNA turnover (27).

Cells treated with anti-Ig for 24 hr are severely inhibited in their ability to synthesize proteins. In contrast, they appear to have virtually a full complement of messages, as judged by translation in a cell-free system. Thus, these cells are apparently under some form of translational control. The site of this regulation remains to be determined. Some proteins appear to be more resistant to this inhibition. One band of ≈35 kDa may be of interest, since a nuclear glycoprotein of similar size (35 kDa) was detectable specifically in resting B cells (28).

It is not clear precisely what immunological event is modeled by this WEHI 231 system. It may be analogous to the induction of self-tolerance among early B cells. In this case, a drop in *c-myc* message levels may result in the immunological suppression of self-reactive B cells. We have noted that the inhibition is reversible at lower concentrations of antibody. Thus, as reported previously (9), one can achieve a cessation of growth, without cell death. Recent models of

tolerance would predict such a phenomenon (29). Alternatively, the data also seem compatible with a two-step B-cell activation model: presentation of antigen (or anti-slg treatment) renders the cells receptive to a second signal. In the absence of this second signal, the cells either enter a quiescent state or die. Although clearly other factors and signals are involved in the regulation of a normal B cell, the WEHI 231 cell system, by permitting detailed observations of cellular responses to individually delivered signals, allows us to begin to address questions concerning the mechanisms by which receptor-mediated growth regulation is effected.

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